sarcomere shortening and slowing relaxation at pH 7.4 while maintaining normal contractile function under acidic conditions. To elucidate this further, double and triple mutants were analyzed to determine the importance of each of the three residues to baseline contractility. The data show that H173 and Q157 are necessary for the reduced contractility at baseline while E166 exerts the opposite effect. Taken together, these new findings suggests that the conformation of helix 4 in TnI is an important determinate of contractility in ischemia.

2848-Pos Board B618
Small Residue Substitutions at Cardiac Troponin I Ser43/Ser45 Produce Distinct Functional Responses
Sarah E. Kampert, Tamara K. Stevenson, Ryan P. O'Connell, Gail L. Romanchuk, Margaret V. Westfall.
University of Michigan Medical School, Ann Arbor, MI, USA.
Our current studies examine the modulatory role of the protein kinase C (PKC)-targeted Ser 43/45 cluster in cardiac troponin I (cTnI). Previously, gene transfer and sarcomeric replacement of endogenous cTnI with phospho-mimetic Asp substitutions at Ser43/Ser45 significantly reduced the peak amplitude and transiently decreased the rate of shortening. Myocytes expressing cTnISer43/45Asp developed an accelerated time to 75% relengthening and Ca^{2+} decay rate. In the current study, we tested whether cTnISer43/45 substitution with Ala would prevent the peak amplitude and shortening rate changes observed with cTnISer43/45Asp. Western blot and immunohistochemistry confirmed that cTnISer43/45Ala gene transfer in adult rat myocytes produced a time-dependent replacement of endogenous cTnI and sarcomeric expression of this cTnI substitution. Unexpectedly, the amplitude and rate of peak shortening in myocytes expressing cTnISer43/45Ala fell between values observed in myocytes expressing endogenous cTnI and cTnISer43/45Asp. These results indicate that additional amino acid properties may be functionally important for these cTnI residues. Next, a cTnISer43/45Asn construct was utilized to test whether polar residues such as Asn serve as a functionally conservative substitution for Ser at these positions. The temporal increase in endogenous cTnI replacement with cTnISer43/45Asn was comparable to cTnISer43/45Ala based on Western analysis and immunohistochemical labeling of myocytes. In ongoing studies, peak shortening amplitude and rate are comparable to controls in myocytes expressing cTnISer43/45Asn, unlike cTnISer43/45Ala. At present, these recent results support the idea that properties other than residue size at Ser43/45 may be important for mimicking the basal shortening phenotype.

2849-Pos Board B619
Upregulation of Restrictive N-Terminal Truncation of Cardiac Troponin T in Ischemia-Reperfusion Preserves Ventricular Function
Han-Zhong Feng, Heidi L. Lujan, Karin Przyklenk, Stephen E. DiCarlo, J.-P. Jin.
Department of Physiology and Cardiovascular Research Institute, Wayne State University School of Medicine, Detroit, MI, USA.
The NH2-terminal variable region of cardiac troponin T (cTnT) is restrictively removed in myocardial ischemia-reperfusion. Over-expression of the NH2-terminal truncated cTnT (cTnT-ND) showed a compensatory effect on cardiac function in transgenic mice. Here we further investigated the production of cTnT-ND following in vivo ischemia-reperfusion. Ischemia of 45 min and reperfusion of 3 or 2 hours was produced in vivo in adult pig and mouse hearts. In both models, viable versus necrotic myocardium was delineated by tetrazolium staining. Four isoforms of cTnT were found in the adult pig cardiac muscle. cDNA cloning and sequencing revealed their difference in the NH2-terminal variable region. An increase of fragmented cTnT was found in the salvaged and viable, previously ischemic region of the infarcted pig hearts as detected using a monoclonal antibody (mAb) recognizing the middle region of cTnT. The four cTnT isoforms with NH2-terminal variations produced cTnT fragments of identical size. A mAb raised against the NH2-terminal peptide of cTnT recognized intact but not the fragmented pig cTnT, indicating an NH2-terminal truncation. Myocardial ischemia-reperfusion in conscious mice produced abnormal EKG and reduced ventricular contractile function, accompanied by high levels of cTnT-ND in viable, previously ischemic myocardium. The results demonstrated that the production of cTnT-ND in salvaged cardiac muscle by posttranslational modification is an active and potentially adaptive response to ischemia/reperfusion rather than a consequence of cardiomyocyte death. This concept is supported by enhanced recovery of cardiac function from ischemia-reperfusion in transgenic mice with cardiac over-expression of cTnT-ND versus wild-type controls. These results suggest that the restrictive NH2-terminal truncation of cTnT is a novel posttranslational mechanism that preserves ventricular function in response to myocardial energetic crisis and provides a potential therapeutic target.

2850-Pos Board B620
Toad Cardiac Muscle Utilizes Solely Slow Skeletal Muscle Troponin T Corresponding to Resistance to Ventricular Afterload
Han-Zhong Feng, J.-P. Jin.
Department of Physiology, Wayne State University School of Medicine, Detroit, MI, USA.
Three homologous genes have evolved encoding the vertebrate cardiac, slow skeletal and fast skeletal muscle isoforms of troponin T (ssTnT). In contrast to all other species studied to date, the cardiac muscle of toad (Bufo) contains no cardiac TnT but solely ssTnT. Western blots using monoclonal antibodies against different TnT isoforms demonstrated that cardiac muscles of fish, amphibian, reptile, avian and mammalian species all express exclusively cardiac TnT except for the toad heart that expressed only slow skeletal muscle TnT, but with normal cardiac isoforms of troponin I, tropomyosin and myosin. Using RT-PCR and 3'- and 5'-RACEs on toad cardiac mRNA templates and degenerated primers derived from the conserved region of slow TnT, we cloned full length cDNAs encoding two isoforms of slow skeletal muscle TnT, of which the exon 5 encoded segment was alternatively spliced. Expression of the cloned cDNA in E. coli showed protein products with apparent molecular weights same as that of the high and low molecular weight TnT isoforms found in the toad cardiac muscle. The sequencing results confirmed that the toad cardiac muscle expresses solely slow skeletal muscle TnT. Functional studies of isolated working toad and frog hearts found that the toad hearts exhibited significantly higher resistance to afterload. Therefore, the unique evolutionary selection of ssTnT in toad cardiac muscle suggests a fitness value. The utilization of ssTnT in toad heart may be an adaptation to improve systolic function. The experimental data demonstrated the physiological significance of the fiber type-specific TnT isoforms and TnT may be a potential target for the improvement of systolic function of the heart.

2851-Pos Board B621
Alterations in Hyper-Variable N-Terminal Region of Cardiac Troponin T Result in Diminished Cardiac Myofilament Function
Sampath K. Gollapudi, Ranganath Mamidi, Srilakshmi Mallampalli, Murali Chandra.
Washington State University, Pullman, WA, USA.
Cardiac troponin T (cTnT) plays a central role in Ca^{2+}-dependent regulation of myofilament activation in cardiac muscle. cTnT is characterized by its unique hyper-variable N-terminal extension (T1) that is rich in negative charge when compared to skeletal TnT (sTnT) isoforms, implying that T1 has a cardiac-specific role in Ca^{2+}-based myofilament activation. Studies have shown that alterations in T1 affect the overall confirmation of cTnT and the binding affinity of cTnT to Tropomyosin, Troponin C, and Troponin I. To examine the unique role of T1 on cardiac activation, we created transgenic (TG) mice expressing recombinant chimeric form of TnT, where mouse $cTnT_{1\text{-}73}$ amino acid residues were replaced by mouse fast $sTnT_{1\text{-}41}$ residues resulting in a less acidic isoform of TnT. Papillary muscle fiber bundles isolated from TG and wild-type (WT) mouse hearts were used to measure tension, ATPase activity, myofilament Ca^{2+}-sensitivity (pCa_{50}), rate of tension redevelopment (k_{tr}), and crossbridge recruitment dynamics at sarcomere length (SL) of 1.9 and 2.3 μm. Maximal tension and ATPase activity were unaltered in fiber bundles from TG and WT mouse hearts. However, an interesting finding was a significant increase in myofilament Ca^{2+}-sensitivity in TG mouse hearts (a pCa_{50} of 5.85 vs. 5.76 in WT mouse hearts). Furthermore, TG mouse hearts exhibited an impaired length-dependent activation, where the length-dependent increase in myofilament Ca^{2+}-sensitivity (ΔpCa_{50}) was 0.04 in TG mouse hearts versus a ΔpCa_{50} of 0.14 in WT mouse hearts. Measurements of k_{tr}, and crossbridge detachment rate at either SL indicated that the crossbridge kinetics was lower in fibers from TG mouse hearts. Thus, all our results demonstrate that structural alterations in T1 of cTnT lead to diminished cardiac function, implicating a regulatory role for T1 in cardiac myofilament activation.

Cell & Bacterial Mechanics & Motility III

2852-Pos Board B622
The Mechanics of Filopodial Retraction
Thomas Bornschlögl.
Institut Curie, Paris, France.
Filopodia are very dynamic, tentacle like cell protrusions that play important mechanical and sensory roles for different cell processes. They are involved in cell migration, wound healing and in the dorsal closure during the embryonic development. Filopodia have been recently observed while actively pulling the invasive bacteria Shigella towards the host cell before infection occurred. In all